ENDOMETRIAL HYPERPLASIA AS OBSERVED IN EXPERIMENTAL GUINEA PIGS

by

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M. S., Kansas State College
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A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1937

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INTRODUCTION

Certain tissues of guinea pigs were studied to find an explanation for failure of reproduction when the animals were fed a diet otherwise adequate but containing only a limited amount of vitamin C, an amount which was, however, adequate for scurvy protection and growth. The studies revealed endometrial hyperplasia in the uteri of these animals, similar in appearance to specimens which various workers observed in human uteri and in experimental guinea pigs, rats, and mice injected with estrogen. These findings pointed to the possibility that vitamin C is a factor causing a similar disturbance. No reference has been made to hyperplasia in connection with diet. The purpose of this study was to investigate the importance of vitamin C in the hypophyseal-ovarian relationship.

REVIEW OF LITERATURE

No literature was found concerning the direct effect of vitamin C on uterine tissue. The transition from the pseudostratified, actively growing, high columnar epithelium of the estrin phase to the low columnar, moderately regular, and quiescent epithelium of the combined corpus luteum and estrin phase was stressed by Burch, Williams and Cunningham (2) in their studies of the human endometrium. They definitely concluded that the histological appearance of the endometrium obtained from cases of Swiss cheese hyperplasia are very similar to those found in animals which have been injected with the estrin hormone.

The experimental production of an excessive endometrial hyperplasia, identical in all macroscopic and microscopic characters with the Swiss cheese hyperplasia of human pathology, which occurred in a castrated adolescent chimpanzee and in a normal mangabey monkey following a course of estrin injections was reported by Zuckermann and Morse (11).

By injecting a series of spayed guinea pigs and rats with varying amounts of estrin over relatively long periods of time, Wolfe, Campbell, and Burch (10) found it possible to produce both glandular and cellular change of hyperplasia in the endometrium in all animals injected.

METHODS

This experiment was based on observations of more than 70 female guinea pigs which were secured from three different colonies known to contain animals of good breeding quality. Many of the females were larger and more mature than the small 300 gm. guinea pigs often used in vitamin C work, especially if vitamin C assay is involved. The animals were cared for in a laboratory which has been used for a number of years and found desirable for guinea pig experimental work. The animals lived in large cages, each accommodating 3 or 4 females. Fresh water and food (vitamin C-free diet) were available at all times.

The modified vitamin C-free diet of Sherman, LaMer, and Campbell (9), fed to all animals, had the following ingredients:

Baked skim milk (baked 2 hours - 250°F.)	30%
Butter fat	9%
Salt	1%
Rolled oats) Wheat bran) ½ and ½ by wt.	59%
Cod liver oil	1%
	100%

The dried skim milk powder furnished a good quality of protein and the minerals, calcium and phosphorus. Heating the skim milk powder at 250°F. for 2 hours removed the vitamin C. Vitamins A and D were provided by the butter fat and cod liver oil. Roughage furnished by the bran and oats was supplemented by generous amounts of filter paper which was kept in the cages at all times. The diet, which has been widely used, was shown in this laboratory, (5) to be devoid of vitamin C but adequate in all other respects for growth and reproduction.

The weights of the females were recorded daily and used to determine the amount of supplement to be fed, and to check the stage of oestrum as the oestrus period is accompanied by a temporary loss in weight. In the care and management of the animals this information concerning body weights was important in securing pregnancy with known copulation age of the embryo. The females were also inspected daily for the presence or absence of the membrane which closes the opening of the vagina during the dioestrous period. A female was placed with the male for breeding when an open vagina indicated the onset of the period of oestrus.

The animals were fed the basal diet and greens until placed on experiment. The females were usually assigned to one of the experimental groups immediately after successful copulation. In a few instances the females continued to receive the vitamin C-free diet plus greens for two weeks before being assigned to an experimental group. This was done to encourage implantation because there had been difficulty in securing pregnancy, even after successful copulation.

Orange juice, frequently used experimentally as a supplement to supply vitamin C, was freshly extracted immediately before the daily feedings. The fresh orange juice was valued at an average vitamin C content of 0.5 mg. per ml. This figure was secured from numerous titrations in this laboratory, following the method of Bessey and King (1) and from average results recently published by Bessey and King (1), Mack et.al., (6), Daniel, Kennedy and Munsell (4) and Richardson, Davis and Sullivan (8). There is, of course, a possibility that the orange juice may also supply some other factor particularly desirable or undesirable to animals on a limited diet. Crystalline ascorbic acid was therefore fed to some animals to provide a supplement without a possible complicating factor. "Cebione" as sold by Merck was used, obtained in the form of 10 mg. tablets, one of which was assumed to be equivalent in ascorbic acid to 20 ml. of orange juice. The animals were fed the two supplements at two levels; 3 ml. and 5 ml. orange juice/300 gm. body weight.

The supplements were fed daily, including Sunday. The orange juice was measured and fed through a graduated pipette, while the ascorbic acid, in tablet form, was fed by inserting the required amount into the mouth of the animal. The animals relished the supplements and consumed them greedily.

Negative controls receiving the basal diet with no vitamin C supplement were kept until death occurred, when autopsy was made and various tissues preserved for later study.

Positive controls receiving the basal diet plus greens ad libitum were maintained and killed after about 49 days gestation, a period which seemed significant in fetal life and comparable to the duration of pregnancy in many animals which received limited supplements of vitamin C.

Most females except the negative and positive controls, were given orange juice or ascorbic acid as supplement when successful copulation indicated the beginning of pregnancy. The experimental groups were:

Group I	Basal diet (no supplement) (negative controls)
Group II	Basal diet + greens ad libitum (positive controls)
Group III	Basal diet + 3 ml. orange juice/300 gm. body weight
Group IV	Basal diet + ascorbic acid =0= 3 ml. orange juice/300 gm. body weight
Group V	Basal diet + 5 ml. orange juice/300 gm. body weight
Group VI	Basal diet + ascorbic acid =0= 5 ml. orange juice/300 gm. body weight

The animals were killed when bleeding from the vagina suggested approaching abortion or the reoccurrence of the oestrus cycle after an irregular interval indicated possible resorption. Autopsies were made and the uterus preserved in Bouin's fixing fluid. Transverse sections 8 - 12 microns thick were made of each horn of the uterus and stained in Ehrlich's hematoxylin.

EXPERIMENTAL RESULTS

Group I (Negative Controls)

The negative controls receiving no vitamin C supplement developed scurvy and died after a period of about 33 days. Marked vaginal bleeding was noted in a number of these animals. Although they were on experiment for a period extending over more than two oestrus cycles, with only one exception, they showed no indications of the oestrus cycle, that is, no open vagina.

Microscopic examinations of the uteri revealed a definite pattern of endometrial hyperplasia (fig. 1). The glands were dilated irregularly, the epithelial lining was high columnar and pesudostratified indicating the effect of estrin. The cells of the stroma were small, compact, and appeared shrivelled. The glands, which were usually large, tortuous and numerous, contained secretions or coagulated serum (fig. 4).

Group II (Positive Controls)

The endometrium of these animals, in contrast to those receiving deficient amounts of vitamin C, contained small glands which were not dilated (fig. 2). The cells of the epithelial lining were low columnar and stratified. The stroma was loosely knit, the cells were large and well filled with protoplasm (fig. 2 and fig. 3). These characteristics indicated the predominance of the corpus luteum hormone.

Group III

This group consisting of those receiving orange juice at the rate of 3 ml./300 gm. body weight, did not give birth to young. When the vitamin C intake was limited, they responded in several ways (table 1). Following successful copulation, S-140, S-80 and S-128 showed no signs of pregnancy but had open vaginas and recurring oestrum after the regular interval. When they were killed after the second oestrus period, no sign of pregnancy was apparent. The remaining four (S-94, S-106, S-42, S-104) gained in weight with no return of the oestrus cycle until 28 - 31 days of gestation. At this time an open vagina, which in most cases was accompanied by vaginal bleeding, indicated that pregnancy was not proceeding normally. In all cases, dead embryos in the process of resorption were found when the animals were killed.

Microscopic examination of the endometrium revealed dilation of the glands, and the presence of secretions. The epithelial lining was high columnar and pseudostratified. The stroma cells were irregular, small and heaped, giving the appearance of being blurred. This presented a typical picture of endometrial hyperplasia.

Group IV

Of those animals fed crystalline ascorbic acid at the rate of 5 ml./300 gm. body weight, four were non-pregnant when killed, S-112 was aborting at 25 days of gestation and S-98 was resorbing the embryos after 25 days of gestation. S-74 gave birth to young. Those which were non-pregnant showed hyperplasia by a distinct alteration of the histological character of the endometrium. The epithelial lining was pseudostratified in each case, the glands contained secretions, and the stroma cells were small and compact. In one case the stroma was edematous. The glands in the endometrium of the aborting animal (S-112) were greatly dilated and the stroma was characteristically hyperplastic, as shown in figure 5. In the uterus of S-98, embryos in the process of resorption were found in one horn, while the lower horn was sterile. In the fertile horn the glands were not greatly dilated nor the stroma very compact. The high columnar pseudostratified epithelium, however, was characteristic of endometrial hyperplasia (fig. 7). The sterile horn contained dilated glands and the characteristic hyperplastic stroma (fig. 6). The hyperplasia in this animal, however, was not as severe as in the one where resorption was occurring. In these two groups receiving supplement at the rate of 3 ml. orange juice/300 gm. body weight, gestation progressed for an average period of 28 days before bleeding from the vagina indicated complications.

Group V

In this group of 7 guinea pigs receiving orange juice as supplement at the rate of 6 ml./300 gm. body weight, only 1 female was not pregnant while 2 females produced living young during the course of the experiment. For S-76 and S-78, an average of 42 days of gestation elapsed before difficulties arose, and bleeding was less frequent than in previous groups. As shown in table 1, S-100 and S-84 were in fair condition, containing live embryos when they were killed at 40 days gestation, while S-88 produced two young, one of which was dead at birth while the other lived 2 weeks. S-76 gave birth to 4 young; was bred again and was pregnant 36 days before a bleeding vagina indicated complications. These better results indicate that with larger amounts of vitamin C, breeding conditions are improved.

Hyperplasia was evident in each case, showing that reproductive conditions were not normal but better than those with only 3 ml. orange juice/300 gm. body weight intake of vitamin C.

Group VI

Of the animals in Group VI, receiving ascorbic acid equivalent to 5 ml. orange juice/300 gm. body weight, all females became pregnant, 2 females had live embryos after 40 days of gestation while 3 females had dead embryos. Only 1 female was observed to have a bleeding vagina, this occurring after 34 days of gestation (table 1). S-122 had received ascorbic acid supplement since birth. This vitamin C supplement, equivalent to 5 ml. orange juice/300 gm. body weight was enough for growth and early pregnancy, but insufficient for reproduction, for when the female (S-122) was killed after 13 days of gestation, the 4 embryos found were dead. However, S-64 produced 2 litters of live young which thrived and grew. One young of the first litter (S-122) became pregnant, as mentioned above.

Hyperplasia was present in the uterine tissues of this group of animals but dilation of the glands was less than in those receiving smaller amounts of vitamin C supplement.

The results from Groups V and VI, each receiving vitamin C at the rate of 5 ml. orange juice/300 gm. body weight, show that although this intake of vitamin C is not adequate for normal and successful reproduction, it is decidedly better than the lower intake of 3 ml. orange juice/300 gm. body weight.

Table I. Summary showing distribution of guinea pigs

into experimental groups.

Group I. (Negative controls - Receiving no vitamin C supplement.

Animal	Days without supplement	Experiment began at oestrus	Open vagina during experiment	Open vagina no. times	Weight at copulation	Notes
N-1	14				-	Killed before marked scurvy developed.
N-6	14				-	Killed before marked scurvy developed.
S-142	29	x			353	Killed as death from scurvy was expected.
S-114	32	x			433	Died of scurvy.
S-134	34				302	Died of scurvy.
S-6	37		x	1	370	Died of scurvy.
O-4	45				-	

(Table 1 - continued)

Group II. (Positive controls) - Receiving greens ad libitum.

Animal	Received supplement before copulation	Open vagina during exper-iment	Open vagina no. times	Killed after copulation no. days	Weight at copulation	Notes
H-8	x			5	-	Pregnancy progressed normally
H-1	x			6	-	Pregnancy progressed normally
I-7	x			6	-	Pregnancy progressed normally
J-32	x	x	1	50	656	In good condition
J-39	x			56	777	In good condition

(Table 1 - continued)

Group III. Receiving orange juice 3 ml./ 300 gm. body weight.

Animal	Received supplement: before copulation no. days	Received supplement: day of copulation	Killed after copulation no. days	Open vagina: during experiment	Open vagina: no. times	Weight at copulation	Notes
S-140	16		30	x	1	330	Not pregnant
S-30	30		50	x	2	560	Not pregnant
S-120		x	35	x	1	540	Killed - difficulty obvious - not pregnant
S-04		x	25	-		300	Bleeding - resorbing
S-106	62		30	x	1	465	5 embryos - all dead
S-42		x	32	x	1	435	3 embryos - all dead
S-104		x	30	x	1	414	3 embryos - 2 dead 1 alive

(Table 1 - continued)

Group IV. Receiving ascorbic acid equivalent to 5 ml. orange juice/300 gm. body weight.

Animal	Received supplement day of copulation	Killed after copulation no. days	Open vagina during experiment	Open vagina no. times	Weight at copulation	Notes
S-30	x	35	x	1	743	Not pregnant
S-90	x	65	x	5	454	Not pregnant
S-96	x	32	x	2	427	Not pregnant
S-180	x	17	x	1	318	Not pregnant
S-98	x	33	x	1	440	3 embryos - all dead
S-112	x	36	x	1	310	Aborting - 1 embryo dead
S-74	x	97			761	2 live young

(Table 1 - continued)

Group V. Receiving orange juice 6 ml./300 gm. body weight.

Animal	Received supplement				Killed after copulation no. days	Open vagina		Weight at copulation	Notes
	no. days	before copulation no. days	day of copulation	after copulation		during experiment	no. times		
S-110	90				90	x	2	-	Not bred
S-60	40			15	55	x	3	501	Not pregnant
S-76	34			15	40	x	3	544	2 embryos - dead - 35 days development
S-70		63			56	x	1	432	Embryos - dead
S-100			x		40			530	3 live embryos
S-84	37			15	52			441	2 embryos alive
S-50				15	140	x	4	535	Produced young

(Table 1 - continued)

Group VI. Receiving ascorbic acid equivalent to 5 ml. orange juice/500 gm. body weight.

Animal	Received supplement no. days	Received supplement before copulation no. days	Received supplement day of copulation	Received supplement after copulation	Killed after copulation no. days	Open vagina during experiment	Open vagina no. times	Weight at copulation	Notes
S-124	90					x	5	-	Never bred
S-122	90	47			13	x	5	405	4 embryos - dead
S-116			x		34			460	3 embryos - dead
S-44				15	40			440	4 embryos - one badly resorbed
S-92			x		40			350	2 embryos - alive
S-36	35			15	50			555	5 embryos - alive
S-64	172			15				600	Produced 2 litters

DISCUSSION

Endometrial hyperplasia, previously associated with an unbalance of the hypophyseal-ovarian relationship and produced experimentally by injection of estrogen, (2,11) has been observed in the tissues of guinea pigs which received limited amounts of vitamin C. This condition has not heretofore been considered in connection with the adequacy of the diet.

In animals which naturally run irregular oestral cycles, Burch, Wolfe, and Cunningham (3) found cystic ovaries and hyperplastic changes of endometrium. They concluded that the condition appears spontaneously in rodents as well as in women. Thus it appears possible to cause such a condition by a disturbing factor which in this case is vitamin C.

There is a question whether there is a real excess or only an apparent excess of estrin, due to a failure of the corpus luteum according to Burch, Williams, and Cunningham (2). This experiment suggests an apparent excess and may explain why with an increase of vitamin C the hyperplasia is less severe.

Bleeding which often occurs in Swiss cheese hyperplasia (2) and the return of the oestrus cycle during pregnancy, detected by an open vagina indicating the effect of estrin, corresponds with the assumption that there is a disturbance in the hormone relationship and points to the similarity of this condition and the hyperplasia produced experimentally (2,3).

When the daily examination of the pregnant animals revealed vaginal bleeding, the animals were killed to prevent any risk of losing the results of the experiment. When abortion occurs the fetuses are often destroyed if the situation is not discovered at once.

The differences noted in the two horns of the uterus when only one contained embryos is consistent with the findings of Parkes (7). He reported that if one horn of the uterus of the mouse is naturally or artificially devoid of fetuses while the other horn is pregnant, then large distended glands appear in the sterile horn during the last week of pregnancy. In this experiment the glands were dilated in the sterile horn in each case (3-30, 3-32, 3-34, 3-70, 3-100). Apparently the difference between the two horns existed through much of the period of gestation, because it was found in animals killed after 23, 36, 40, and 52 days of pregnancy. The comparison of the two horns is illustrated in figures 6 and 7.

The hyperplasia seemed to be associated with pregnancy. In each case where the animals failed to become pregnant after copulation, the stroma cells were compact and the epithelial lining was pseudostratified but the glands showed less dilation (fig. 4).

CONCLUSIONS

1. Endometrial hyperplasia, previously associated with an unbalance of the hypophyseal-ovarian relationship and produced experimentally by injection of estrogen, has been observed in tissues of guinea pigs which received limited amounts of vitamin C.
2. Increasing the amount of vitamin C supplement beyond the level of 3 ml. orange juice/300 gm. body weight (an amount often regarded as adequate for the guinea pig), appeared to decrease the severity of the hyperplasia and lengthen the duration of gestation.
3. The hyperplasia seemed to be associated with pregnancy. It was less pronounced in those animals whose uteri contained no embryos.
4. When embryos were found in only one horn of a pregnant guinea pig receiving limited amounts of vitamin C, the dilation of the glands was less pronounced in the fertile horn than in the sterile horn.

ACKNOWLEDGMENT

The writer wishes to express her sincere appreciation to her major instructors, Dr. Martha M. Kramer, Professor of Food Economics and Nutrition, and to Dr. Mary T. Harman, Professor of Zoology, for their helpful advice and assistance in all matters pertaining to this study.

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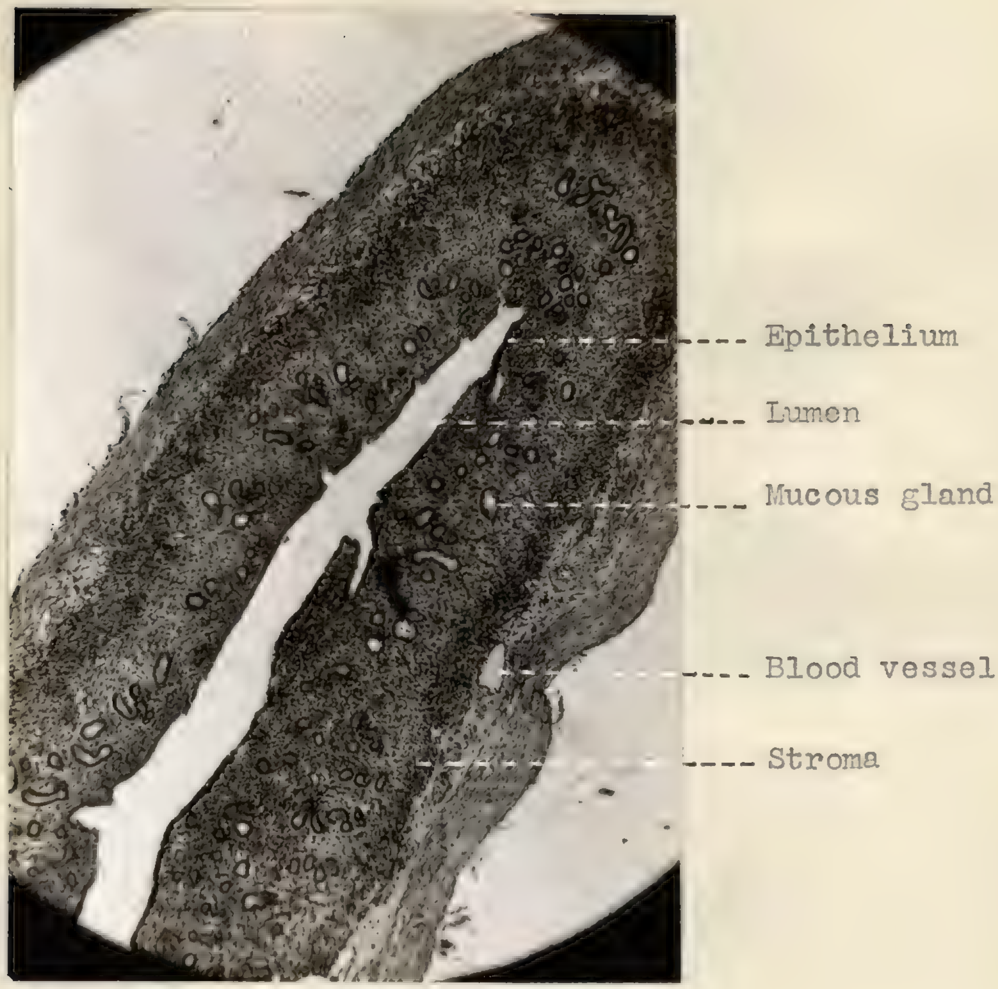

Fig. 1. Photomicrograph of section from uterus of guinea pig H-I which received no vitamin C supplement (negative control). Note the compact stroma and dilated glands characteristic of endometrial hyperplasia.

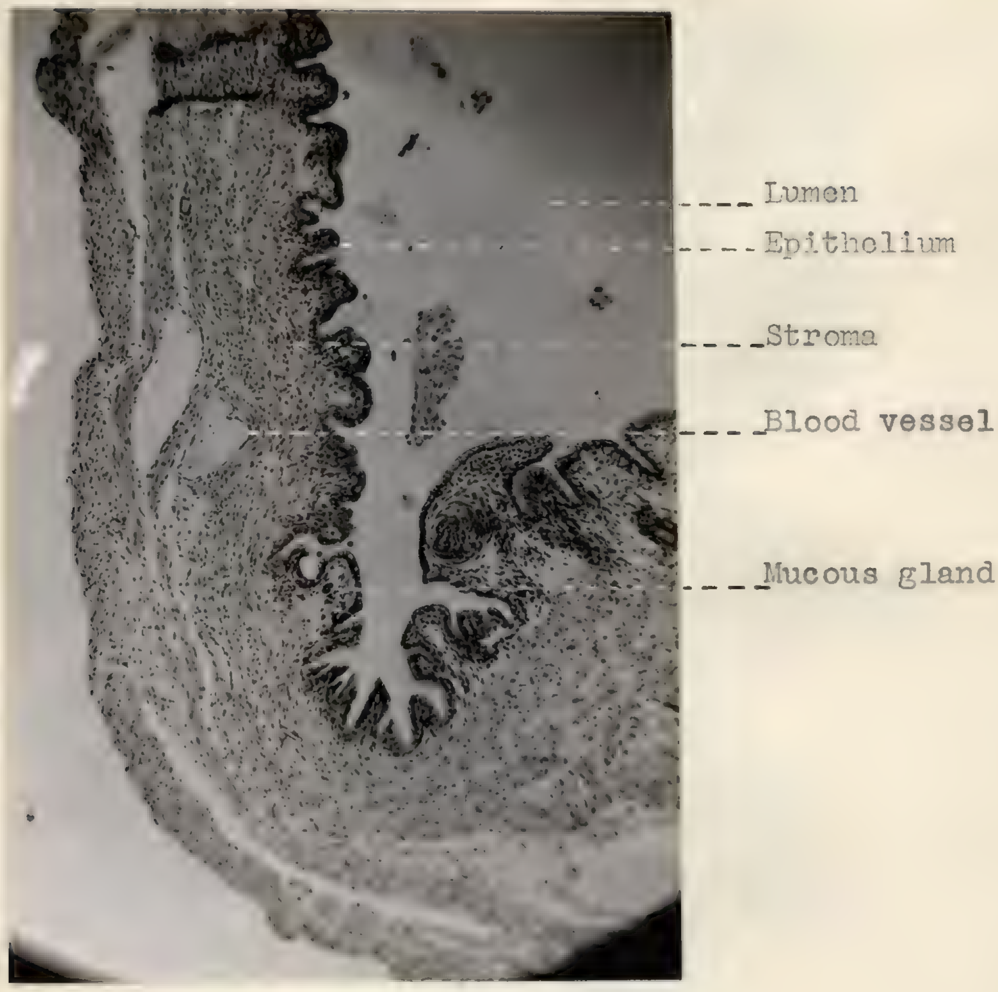

Fig. 2. Photomicrograph of section from uterus of guinea pig S-62 which was pregnant 35 days and received ample supplements of vitamin C (positive control). The glands are less numerous, small, and not dilated. The stroma cells are large.

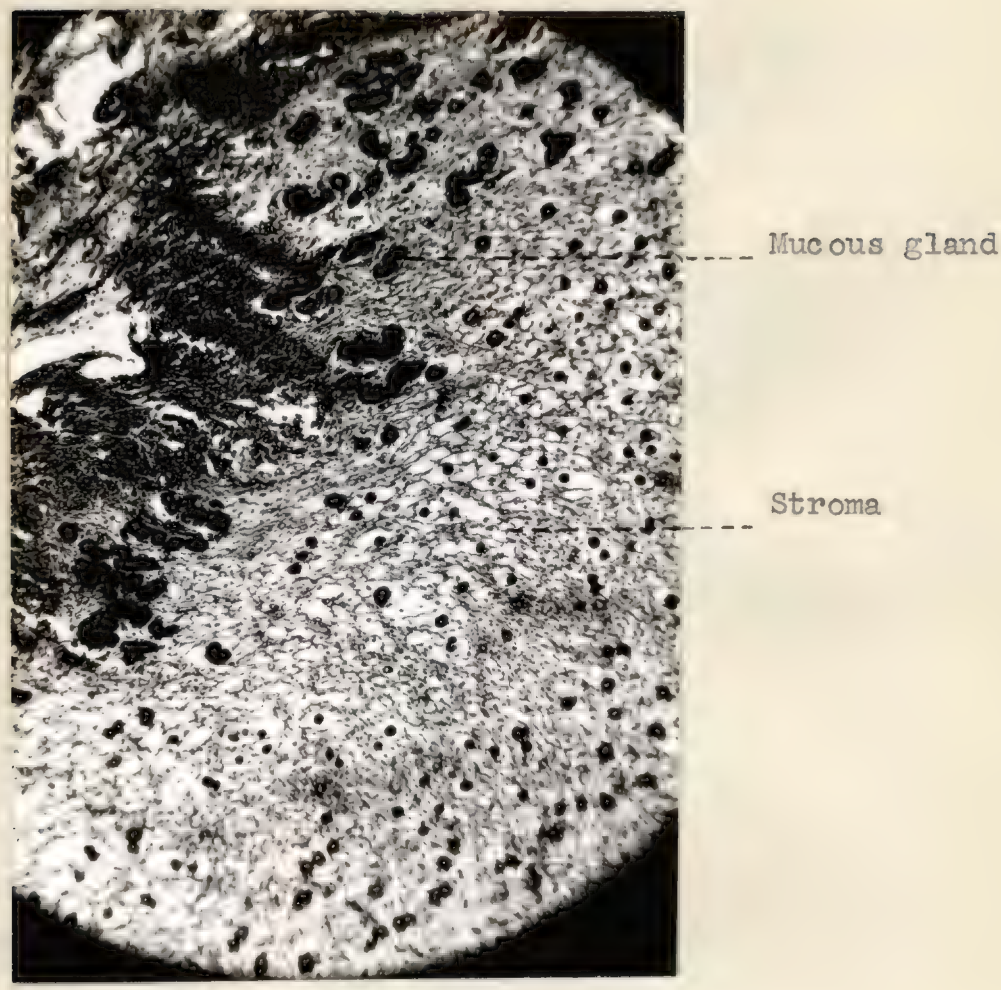

Fig. 3. Photomicrograph of section from uterus of guinea pig M-2 which was pregnant 8 days. Note the small glands without dilation and the loosely knit stroma.

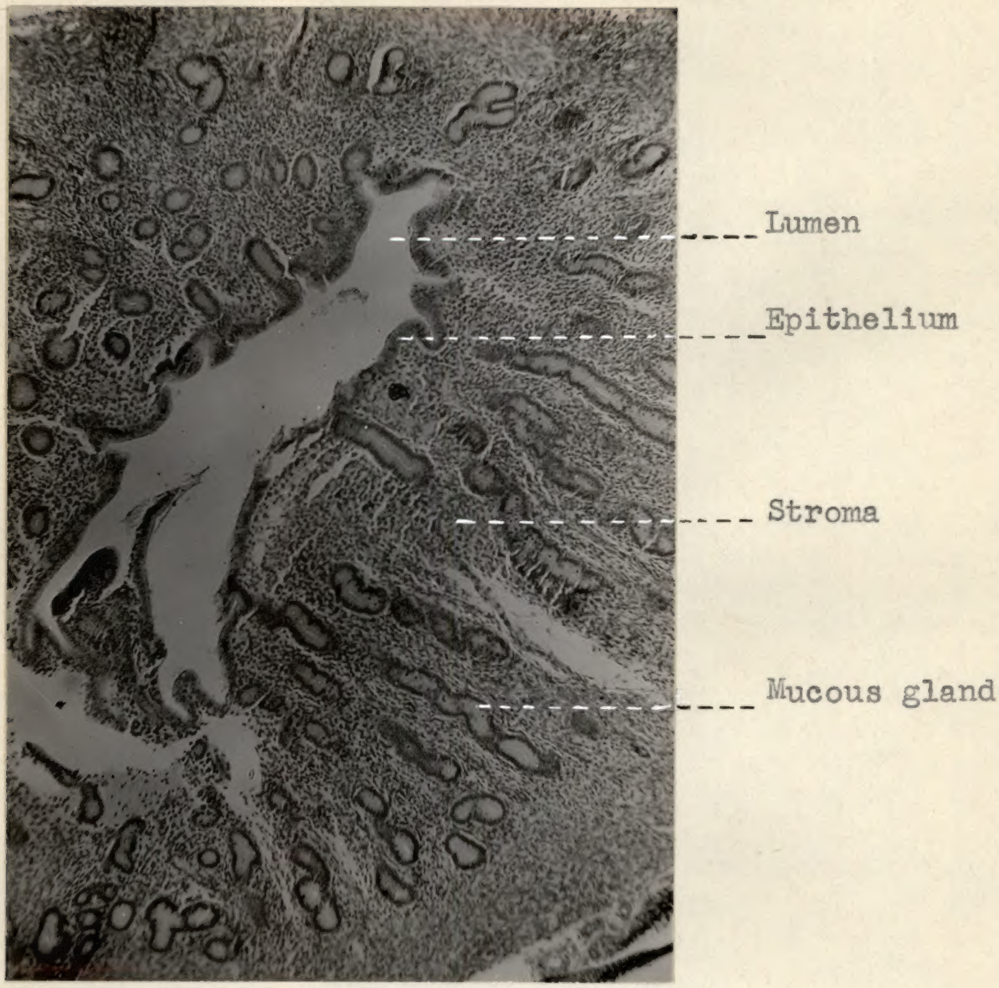

Fig. 4. Photomicrograph of section from uterus of non-pregnant guinea pig S-90 which received ascorbic acid at the rate of 3 ml./300 gm. body weight. Note glands are numerous, large, and tortous.

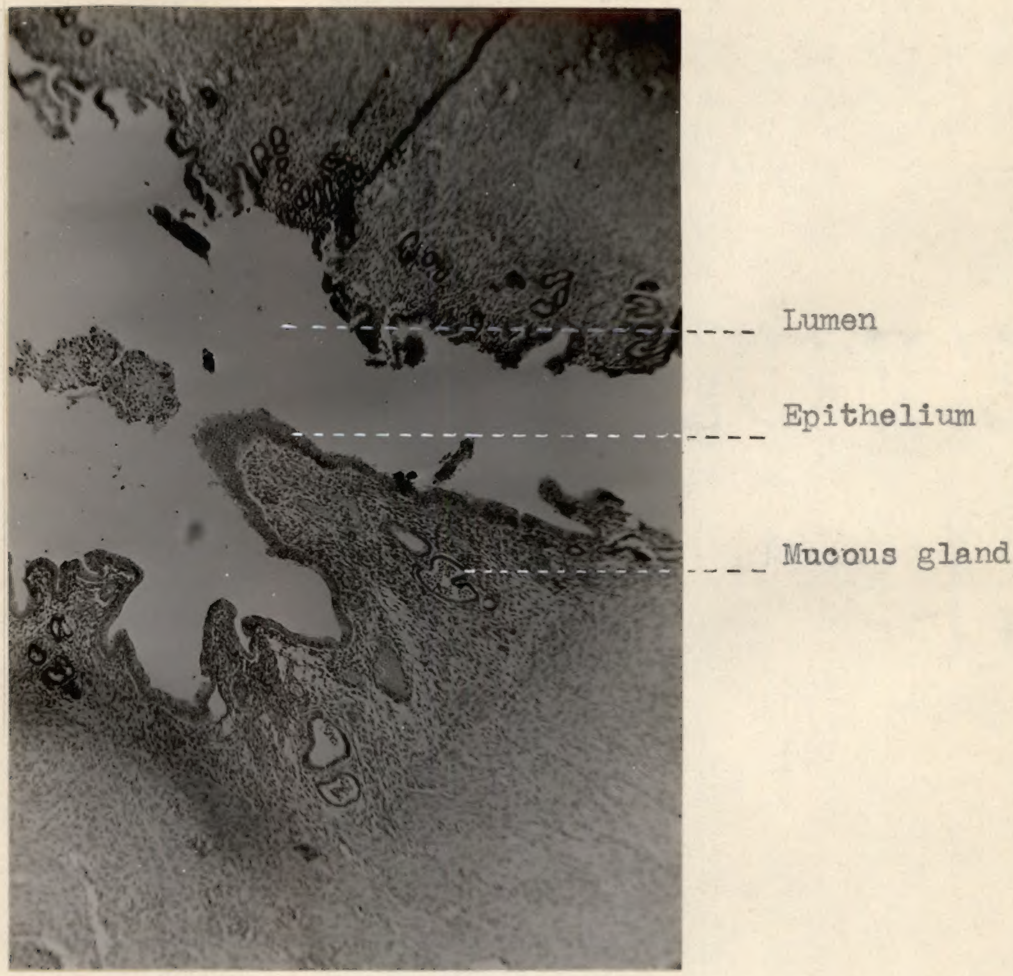

Fig. 5. Photomicrograph of section from uterus of aborting guinea pig S-112 which received ascorbic acid supplement at the rate of 3 ml./300 gm. body weight. Note the greatly dilated glands contain secretions.

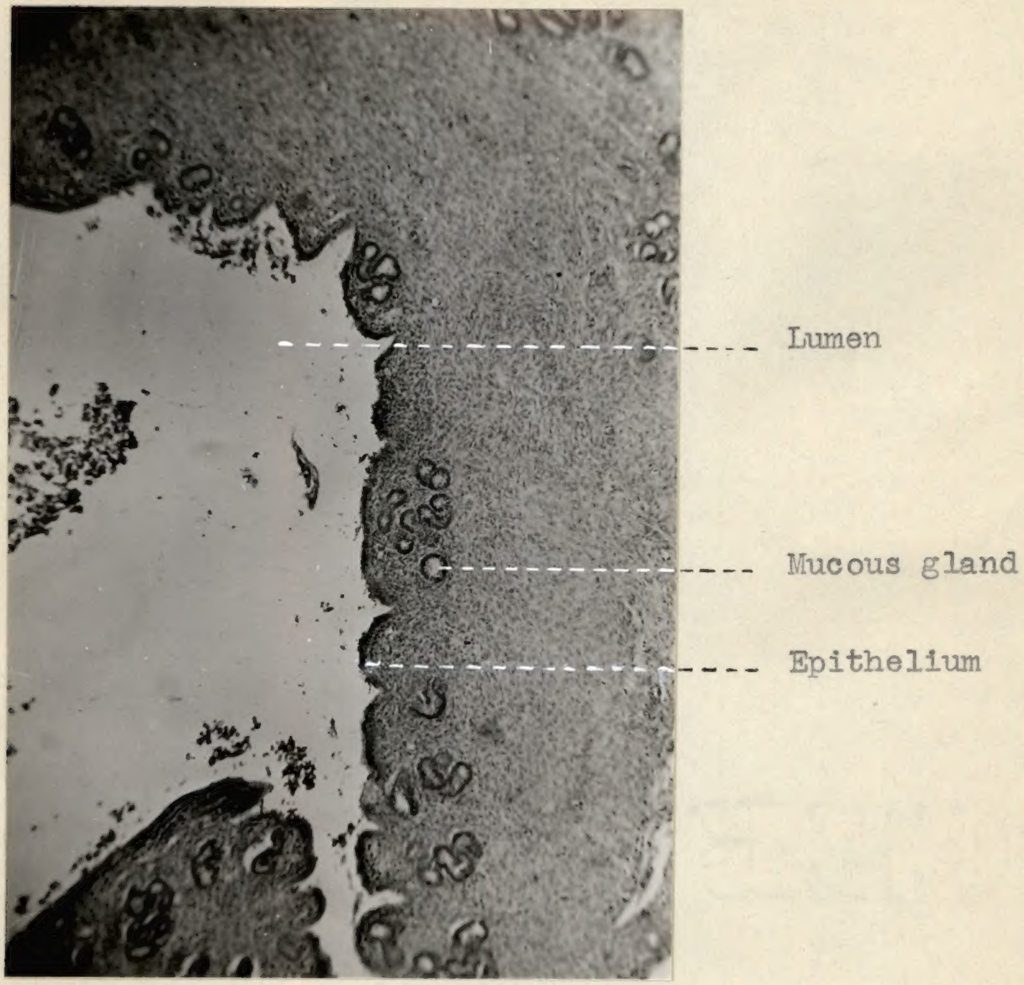

Fig. 6. Photomicrograph of section of sterile horn of guinea pig S-100. The glands are numerous and dilated.

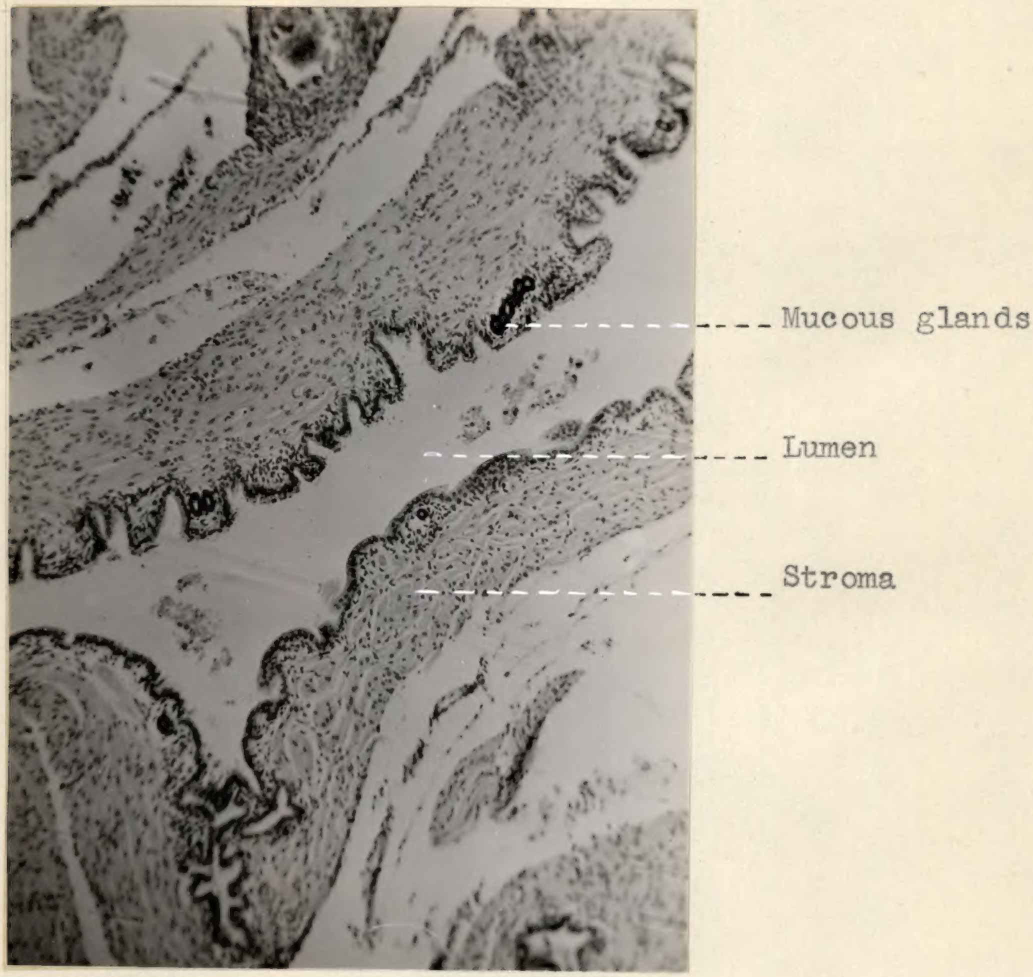

Fig. 7. Photomicrograph of section of fertile horn of guinea pig S-100. Note glands are small, not dilated, and fewer in number.